KTR

Final Report

TBR-0006-14

Silver Ion Sterilizer

Eye irritation test of silver ion sterilizer on rabbits

President of Korea Testing & Research Institute

Study Overview

Study Title: Eye irritation test of silver ion sterilizer on rabbits

Study Number: TBR-0006-14

Sponsor:

Name: CNL Co. Ltd.

Location: 1 Yeonseoidaegil, Heungeupmyeon, Wonju City, Gangwon-do (#304 Business Start-up and Childcare Center for the Disabled)

CEO: KIM, Kyeong Su

Person in charge: SONG, Cheoul Ki Position: Executive director

Contact: Tel. 033-764-2116 Fax. 033-764-2117

Test Facility:

Name: Health Care Research Laboratory of the Korea Testing & Research Institute (KTR)

Location: 12-63 Sandan-gil, Hwasun-eup, Hwasun-gun, Jeollanam-do

Operator in charge: PARK, Jong Il

Contact: Tel. 061-370-7810 Fax. 061-370-7777

Test Method:

-Ministry of Food & Drug Safety Announcement no. 2014-136 (July, 30, 2014), "Toxicity Test Standards for Drugs etc.", [Appendix 8] Eye irritation test

LEE Jin Young, B. S. Date

Study Director

※ This is a report for the test substance provided by the sponsor.

Study Staffs

The following study staffs conducted the important testing steps of the study and reported the results thereof according to the standard operating guidelines of the Health Care Research Laboratory, KTR.

Staff in charge of the study:	CHEONG, Myeong Ho/B.S.
Staff in charge of preparing the test substance:	LEE, Jin Young/B.S.
Staff in charge of animal management:	KIM, Yong Wu/B.S.
Staff in charge of quarantine:	LEE, Jin Young/B.S.
Staff in charge of writing the report:	LEE, Jin Young/B.S.

Contents

1. Summary

In order to evaluate eye irritation on rabbits by silver ion water generated from a silver ion sterilizer, we treated rabbits with the test substance, and then observed the mortality rates, general symptoms and weight changes and evaluated eye irritation for 7 days since treating the rabbits with the test substance. The result was as below.

-There were no animals that died due to treating with the test substance during the testing period.

-There were no special abnormal findings in all the animals.

-All the animals showed normal increase of weight during the testing period.

-No eye irritation was observed in the non-washed group or the washed group on day 1, 2, 3, 4, and 7 since treating with the test substance.

-The evaluation result by Draize calculating method: At the first phase evaluation, Index of Acute Ocular Irritation (I.A.O.I) for the non-washed group and the washed group were both '0.0'.

According to the above eye irritation test on rabbits, silver ion sterilizers are considered to be 'non-irritating matters' to both the washing group and non-washed group.

2. Introduction

This study was conducted to evaluate the eye irritation of silver ion sterilizers to rabbits.

This study was conducted with the approval from the Animal Ethics Committee of the Health Care Research Laboratory of the Korea Testing & Research Institute (KTR) based on the Animals Protection Act[enforcement Aug. 14 2014][Regulation no. 12051(partially amended on Aug. 13, 2013) and the Regulations on Animals for Testing[Enforcement Jul. 30, 2013][Regulation no. 11987(partially amended on Jul. 30, 2013)].

2.1 Test Schedule

Study initiation date	: September 30, 2014
Test initiation date	: October 6, 2014
Date of animal adoption	: October 6, 2014
Quarantine and Purification period	: October 6, 2014 ~ October 13, 2014
Date of application	: October 14, 2014
General symptom observation period	: October 14, 2-14 ~ October 21, 2014
Test end date	: October 21, 2014
Final report(draft) submission date	: November 3, 2014
Study end date	: November 4, 2014

3. Materials & Methods

3.1 Test substance and washing agent

3.1.1 Test substance (Figure 5.)

Name of substance : Silver ion sterilizer

Origin of supply: CNL Co., Ltd.

Date of production: September 1, 2014

Installation date: October 6, 2014

Installed device: Silver ion water producing device set 1

Storing condition during test period: room temperature[(1-30)℃], to be used on the same day it is produced

Appearance and state: Transparent liquid when produced as silver ion water

Effective life period: Until silver plates perish (provided by sponsor)

Disposal of remaining test substance: Discard silver ion water and return producing

Notes for treating/disposing: Keep the device from direct contact of water

3.1.2 Washing materials

Name of product: Sterilized physiological saline water (main water used)

Producer: Dai Han Pharm Co., Ltd.

3.2 Fabrication of Test substance

We used the test substance generated from the silver ion sterilizer that the sponsor installed(device) and set(standard values) without additional preparation.

3.3 Analyses on Test substance

We decided not to conduct a separate analysis on the concentration, safety and

homogeneity of the test substance and test substance preparations after discussing with the test sponsor.

3.4 Test specifications

Type and species: Yac:NZW(KBL), Rabbit, SPF

Origin of supply: Cheonan Yonam College (313 Yonam-ro, Seonghwan-eup, Seobuk-gu, Cheonan-si, Chungnam)

Gender and number of animals when adopted : 9 males

Age and weight of animals when adopted : About 3 months old, 1894.3 ~ 2146.8g

Gender and number of animals at the time of application : 9 males

Age and weight of animals at the time of application : About 3 months old, 2192.6~2497.7g

3.4.1 Reason for selecting the test specifications

We selected NZW rabbits since they are widely used in eye irritation tests, and thus there are abundant basic data accumulated that may be used for comparison.

3.4.2 Quarantine and purification

We examined the appearance and health conditions of all the animals when adopting them, and then we observed their weight changes and general health conditions after 8 days of quarantine and purification, and then used the healthy ones for the test.

3.4.3 Entity identification

We marked animal numbers on the left earflaps of the rabbits during the purification period, and on the their right earflaps during the testing period, and also attached ID cards to the cage.

3.5 Raising environment

3.5.1 Number of animals rooms

Rabbit raising room 5

3.5.2 Environmental conditions

Temperature: (20 ± 3)℃

Relative humidity: (50 ± 20) % R.H.

Number of times of ventilation: (10 ~ 20) times/h

Lighting cycle: Bright condition 12 hours (08:00 ~ 20:00)

Dark condition 12 hours (20:00 ~ 08:00)

Illumination: (150 ~ 300) Lux

Cage type: Stainless steel wire cage

Cage size: (470W x 405D x 600H)mm

Capacity per cage: 1 rabbit

The temperature and humidity of the animal room was measured every 30 minutes by an automatic temperature humidity measuring device, and environmental conditions such as the illumination was measured according to the standard operation guidelines. As a result of measuring the environment of the animal room, there were no factors affecting the test.

3.5.3 Feed and drinking water supply

Radiation sterilized feed for rabbits for testing [Purina, Korea] was used as the feed, and R/O water was provided as free drinking water.

3.5.4 Feed and drinking water inspection

We inspected the feed based on the analysis report that had been prepared under regular inspections by the manufacturer of the feed that we received from the feed supplier, and we inspected the drinking water for any effects on the test based on the

regular inspections according to the standard operation guidelines of the Health Care Research Laboratory of Korea Testing and Research Institute, but found none.

3.6. Test method

3.6.1 Group configuration

Test group	Gender	Animal number	Number of animals	Amount applied (mL)	Application route
T1 (Non-washing)	Male	1101-1106	6	0.1	Eye mucous membrane R(testing area), L(no Application)
T2 (Washing)	Male	1201-1203	3		

R: right eye L: left eye

3.6.2 Eye inspection before Application

We checked by unaided eyes that there no abnormalities in the cornea, iris, and conjunctivae of both eyes about 24 before the application.

3.6.3 Application of test substance

After correcting the rabbits' bodies, we took 0.1mL of the test substance and dropped it directly into the right eyes of 9 rabbits, and left the left eyes without application of the test substance as a control group. After the application, we closed the left and right eyes of the rabbits for about 1 second to prevent the test substance from flowing out of the eyes. We then thoroughly washed the eyes of 3 rabbits from the washing group for 20-30 seconds with 30mL of tepid sterilized physiological saline solution, and left the other 6 animals unwashed.

3.7 Observation items

3.7.1 General symptoms

We observed all the animals once a day, and on the 7^{th} day of end of observation, we euthanized them.

3.7.2 Weight changes

We measured the weights of the animals when adopted, just before applying the test substance, and on the 7th day of end of observation.

3.7.3 Observation on eye reaction

Having the other eye(left eye) as the control group with no application of the test substance, we applied the test substance to the right eyes, and then observed eye irritation in the cornea, iris, and conjunctivae on day 1, 2, 3, 4, and 7 according to [Table A].

3.7.4 Photographing

On day 1 and day 7 since the day of application, we selected one entity from each of the non-washing and washing group that has representativeness, and then attached a label having the test number and the name of test substance, and photographed them.

3.7.5 Evaluation and judgment on eye reactions

The evaluation on eye irritation was conducted according to [Table 4], and we computed the Individual Index of Ocular Irritation (I.I.O.I), Mean Index of Ocular Irritation, and the Index of Acute Ocular Irritation. We evaluated the eye irritation by the test substance in 3 phases according to the following standards.

① Eye irritation Phase 1

We examined the I.A.O.I on day 3 after the application, and checked whether entities showing the observation value within I.A.O.I±5 are 40% or more of the total number of animals.

In this test, we conducted a statistical processing on weight data using an SPSS(Ver 19.0) statistics program. First of all, we conducted an equal variance inspection through a Levene's test, and then a one way ANOVA inspection(variance analysis), but no significance was observed from both male and female rats.

② Eye irritation Phase 2

Based on the I.A.O.I values selected according to the judgment of phase 1, we set the 1st

eye irritation scores according [Table B].

③ Eye irritation Phase 3 (Final judgment)

We gave the final eye irritation grades according to [Table 3] based on the 1st eye irritation grades.

[Table. A] Eye lesion grades

(1) Cornea
(A) Opacity: Degree of thickness of eyeball (observed the most concentrated area) ◦ No suppuration or opacity.....0 ◦ Opacity was dispersed or concentrated (but different from being less transparent) but the end of iris was clearly observable.....1 ◦ Semitransparent area was easily observable but the end of iris was slightly unclear.....2 ◦ Appeared in pearl color and the end of iris was not observable and the size of the pupil was barely observable.....3 ◦ The cornea was non-transparent and the iris was not observable because of opacity.....4 (B) Range of opaque cornea ◦ Less than 1/4 (but not 0).....1 ◦ Not less than 1/4 and less than 1/2.....2 ◦ Not less than 1/2 and less than 3/4.....3 ◦ Not less than 3/4 to 1.....4 Score = A x B x 5 Highest score = 80
(2) Iris
(A) Reaction ◦ Normal.....0 ◦ Significant wrinkles, hyperemia, swelling or moderate hyperemia near the cornea, but the iris reacted to light.....1 ◦ The iris was either not reacting to light, showed hyperemia, or mostly damaged2 Score = A x 5 Highest score = 10
(3) Conjunctiva
(A) Rubefaction (only in palpebral conjunctiva and ocular conjunctiva but not in iris) ◦ Blood vessels normal.....0 ◦ Some of the blood vessels showed hyperemia.....1 ◦ Light crimson color(or crimson color) or each blood vessel was not easily observable.....2 ◦ Dark scarlet color(or scarlet color).....3 (B) Conjunctiva edema ◦ Not swollen.....0

◦ Slightly swollen than normal(including membrane nictitan).....1
◦ Significant swelling along with eversion of eyelid.....2
◦ Eyelid swollen as much as the eye is closed halfway.....3
◦ Eyelid swollen as much as the eye is closed more than halfway.....4
(C) Emissions
◦ No emission.....0
◦ A little emission (more than what is usually seen from the eyes of a normal animal).....1
◦ Emission as much as to wet the eyelashes and eyelids.....2
◦ Emission as much as to wet significant area around the eye and also the eyelashes and eyelids.....3
Score = (A + B + C) x 2 Highest score = 20

[Table 2] Evaluation on I.A.O.I

1st I.A.O.I Grades	Evaluation level
Nonirritating, N	0-0.5
Practically nonirritating, PN	0.5-2.5
Minimally irritating, M_1	2.5-15
Mildly irritating, M_2	15-25
Moderately irritating, M_3	25-50
Severely irritating, S	50-80
Extremely irritating, E	80-100
Maximally irritating, M_x	100-110

[Table C] Standards for Eye Irritation Evaluation phase 3

1st I.A.O.I Grades	Evaluation conditions	Final evaluation grades
Nonirritating, N	Day 1 M.I.O.I. = 0	Nonirritating, N
	Day 1 M.I.O.I. > 0	Practically nonirritating, PN
Practically nonirritating, PN	Day 1 M.I.O.I. = 0	
	Day 1 M.I.O.I. > 0	Minimally irritating, M_1
Minimally irritating, M_1	Day 2 M.I.O.I. = 0	
	Day 2 M.I.O.I. > 0	Mildly irritating, M_2

Mildly irritating, M_2	Day 3 M.I.O.I. = 0	
	Day 3 M.I.O.I. > 0	Moderately irritating, M_3
Moderately irritating, M_3	(1) Day 7 M.I.O.I. ≤ 0 (2) Day 7 I.I.O.I ≤10(60% of total) or no entities of I.I.O.I > 30	
	(1) Day 7 M.I.O.I. >20 (2) Day 7 I.I.O.I >10(60% of total) or existence of entities of I.I.O.I > 30	Severely irritating, S
Severely irritating, S	(1) Day 7 M.I.O.I. ≤ 40 (2) Day 7 I.I.O.I ≤30(60% of total) or no entities of I.I.O.I > 60	
	(1) Day 7 M.I.O.I. > 40 (2) Day 7 I.I.O.I >30(60% of total) or existence of entities of I.I.O.I > 60	Extremely irritating, E
Extremely irritating, E	(1) Day 7 M.I.O.I. ≤ 80 (2) Day 7 I.I.O.I ≤60(60% of total) or no entities of I.I.O.I > 100	
	(1) Day 7 M.I.O.I. > 80 (2) Day 7 I.I.O.I >60(60% of total) or existence of entities of I.I.O.I > 100	Maximally irritating, M_x
Maximally irritating, M_x	(1) Day 7 M.I.O.I. ≤ 80 (2) Day 7 I.I.O.I ≤60(60% of total)	Extremely irritating, E
	(1) Day 7 M.I.O.I. > 80 (2) Day 7 I.I.O.I >60(60% of total)	Maximally irritating, M_x

4. Results

4.1 Mortality and general symptoms (Table 1)

There were no dead animals or abnormal findings caused by the application of test substance during the test period.

4.2 Weight changes (Table 2)

All the animals showed normal increase of weight.

4.3 Observation on applied area (Table 3, Figure 1 -4)

There were no eye irritation in both the washing and non-washing group according to observations made on the application areas on day 1, 2, 3, 4, and 7 since the date of application of the test substance.

5. Discussion & conclusion

In order to evaluate eye irritation on rabbits by silver ion water generated from a silver ion sterilizer, we treated rabbits with the test substance, and then observed the mortality rates, general symptoms and weight changes and evaluated eye irritation for 7 days since applying the test substance to the rabbits.

-There were no animals that died due to application of the test substance during the testing period. All the animals showed normal increase of weight. No eye irritation was observed in both the non-washing group or the washing group. The evaluation result by Draize calculating method: At the first phase evaluation, Index of Acute Ocular Irritation (I.A.O.I) for the non-washed group and the washed group were both '0.0'.

According to the eye irritation phase 1 judgment, I.A.O.I. was '0.0' in both non-washing group and the washing group. According to eye irritation phase 2 judgment, the 1st eye irritation grade was Nonirritating, N. In the final judgment, both the non-washing group and the washing group showed the mean index of ocular irritation (M.I.O.I) of '0.0' on day 1 at the 1st eye irritation grade Nonirritating.

According to the above eye irritation test on rabbits, silver ion sterilizers are considered to be 'non-irritant' matter to both the washing group and non-washed group.

6. References

- Ministry of Food & Drug Safety Announcement no. 2014-136 (Jul. 30, 2014) "Toxicity Test Standards of Drugs etc." [Appendix 8]

- Ministry of Food & Drug Safety Announcement no. 2014-67 (Feb., 12, 2014) "Nonclinical Test Management Standards"

- Ministry of Food & Drug Safety (2012) ""Guidelines to Toxicity Test Standards of Drugs etc."

-OECD Principle of Good Laboratory Practice, ENV/MC/CHEM (98)17 (as revised in 1997)

-Draize J.H., Woodard G. and Calvery H.O. (1944) : Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther., 82:377-390.

7. Tables (Group summary)

Table 1. Mortality and clinical signs

Group	Animal number	Days after application								Mortality
		0	1	2	3	4	5	6	7	
Non-washing	1101	N	N	N	N	N	N	N	N	0/6[a]
	1102	N	N	N	N	N	N	N	N	
	1103	N	N	N	N	N	N	N	N	
	1104	N	N	N	N	N	N	N	N	
	1105	N	N	N	N	N	N	N	N	
	1106	N	N	N	N	N	N	N	N	
Washing	1201	N	N	N	N	N	N	N	N	0/3[a]
	1202	N	N	N	N	N	N	N	N	
	1203	N	N	N	N	N	N	N	N	

N : Normal

[a] : Number of dead animals / Number of total animals

Table 2. Body weight

Unit (g)

Group	Animal Number	Day after application		Weight gains
		DAY 0	DAY 7	
Non-washing	1101	2206.6	2410.9	204.3
	1102	2285.9	2527.1	241.2
	1103	2217.7	2419.6	201.9
	1104	2448.9	2736.4	287.5
	1105	2192.6	2411.1	218.5
	1106	2497.7	2651.8	154.1
	Mean	2308.2	2526.2	217.9
	S.D.	132.7	139.9	44.5
Washing	1201	2310.3	2588.9	278.6
	1202	2260.5	2440.8	180.3
	1203	2353.2	2624.9	271.7
	Mean	2308.0	2551.5	243.5
	S.D.	46.4	97.6	54.9

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Table 3. Evaluation of eye irritation

Group			Non-washing						Washing		
Animal number			1101	1102	1103	1104	1105	1106	1201	1202	1203
Cornea [Score = A×B×5]	Degree of opacity (A)	DAY 1	0	0	0	0	0	0	0	0	0
		DAY 2	0	0	0	0	0	0	0	0	0
		DAY 3	0	0	0	0	0	0	0	0	0
		DAY 4	0	0	0	0	0	0	0	0	0
		DAY 7	0	0	0	0	0	0	0	0	0
	Diffuse areas of opacity (B)	DAY 1	0	0	0	0	0	0	0	0	0
		DAY 2	0	0	0	0	0	0	0	0	0
		DAY 3	0	0	0	0	0	0	0	0	0
		DAY 4	0	0	0	0	0	0	0	0	0
		DAY 7	0	0	0	0	0	0	0	0	0
Iris (A) [Score = A×5]		DAY 1	0	0	0	0	0	0	0	0	0
		DAY 2	0	0	0	0	0	0	0	0	0
		DAY 3	0	0	0	0	0	0	0	0	0
		DAY 4	0	0	0	0	0	0	0	0	0
		DAY 7	0	0	0	0	0	0	0	0	0
Conjunctivae [Score = (A+B+C)×2]	Redness (A)	DAY 1	0	0	0	0	0	0	0	0	0
		DAY 2	0	0	0	0	0	0	0	0	0
		DAY 3	0	0	0	0	0	0	0	0	0
		DAY 4	0	0	0	0	0	0	0	0	0
		DAY 7	0	0	0	0	0	0	0	0	0
	Edema (B)	DAY 1	0	0	0	0	0	0	0	0	0
		DAY 2	0	0	0	0	0	0	0	0	0
		DAY 3	0	0	0	0	0	0	0	0	0
		DAY 4	0	0	0	0	0	0	0	0	0
		DAY 7	0	0	0	0	0	0	0	0	0
	Discharge (C)	DAY 1	0	0	0	0	0	0	0	0	0
		DAY 2	0	0	0	0	0	0	0	0	0
		DAY 3	0	0	0	0	0	0	0	0	0
		DAY 4	0	0	0	0	0	0	0	0	0
		DAY 7	0	0	0	0	0	0	0	0	0
I.I.O.I.[a]		DAY 1	0	0	0	0	0	0	0	0	0
		DAY 2	0	0	0	0	0	0	0	0	0
		DAY 3	0	0	0	0	0	0	0	0	0
		DAY 4	0	0	0	0	0	0	0	0	0
		DAY 7	0	0	0	0	0	0	0	0	0
M.I.O.I.[b]		DAY 1	0.0[c]						0.0[c]		
		DAY 2	0.0						0.0		
		DAY 3	0.0						0.0		
		DAY 4	0.0						0.0		
		DAY 7	0.0						0.0		

[a] : I.I.O.I. (Individual Index of Ocular Irritation) = Score (Cornea + Iris + Conjunctivae)

[b] : M.I.O.I. (Mean Index of Ocular Irritation) = Σ I.I.O.I. / Number of animals

[c] : I.A.O.I. (Index of Acute Ocular Irritation) = The maximum value of M.I.O.I.

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8. Figures

Figure 1. Eye photographs of non-washing group on day 1 after application of test substance

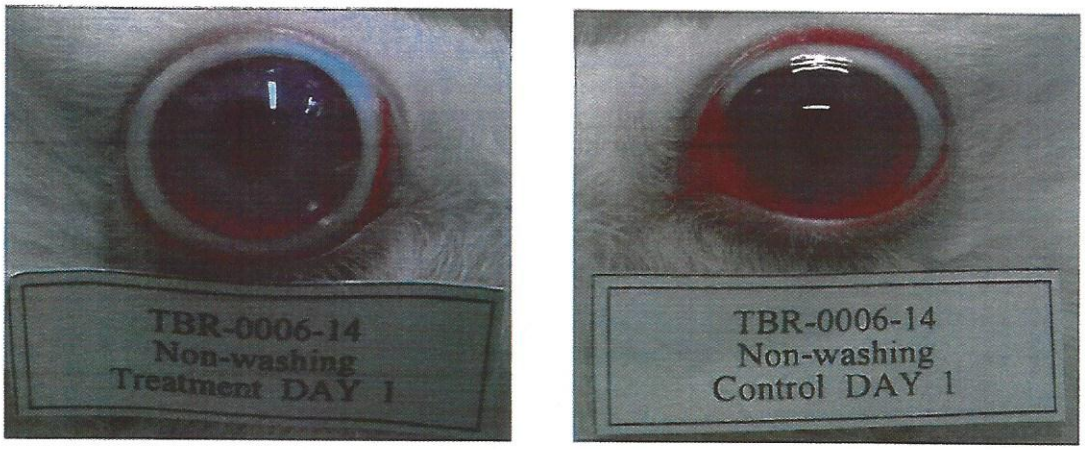

Figure 2. Eye photographs of non-washing group on day 7 after application of test substance

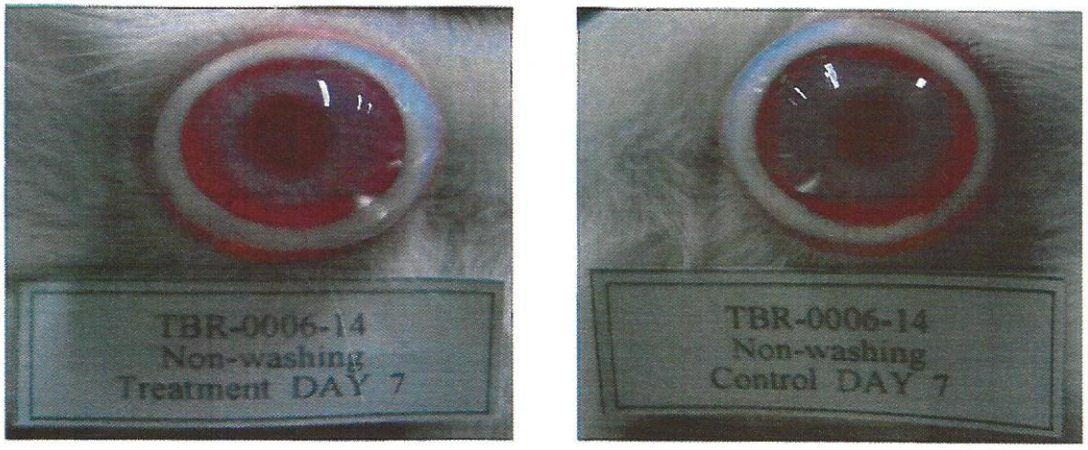

Figure 3. Eye photographs of washing group on day 1 after application of test substance

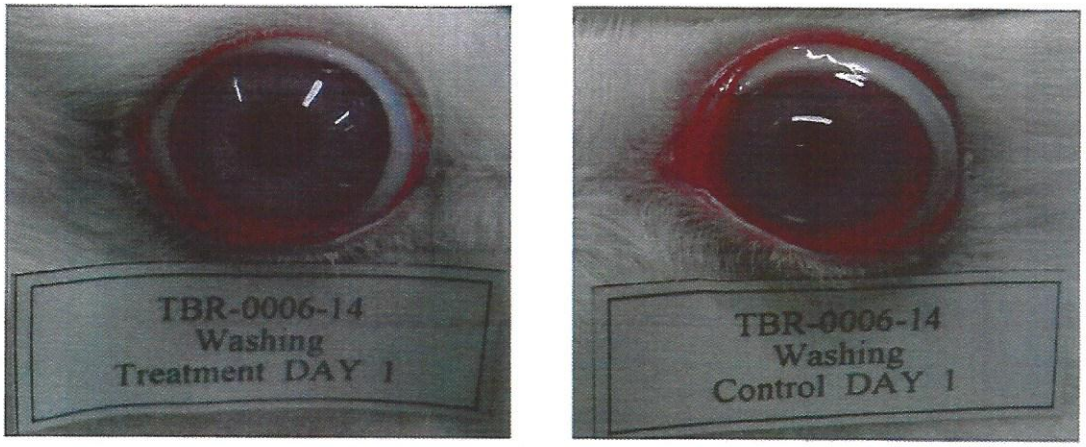

Figure 4. Eye photographs of washing group on day 7 after application of test substance

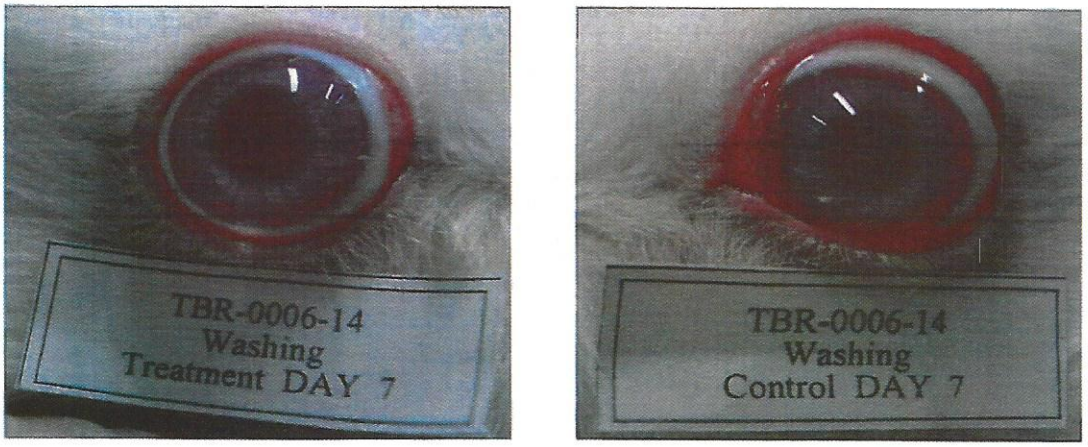

Figure 5. Test substance